

Multiple positive steady states in subnetworks defined by stoichiometric generators

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Abstract

In Systems Biology there is a growing interest in the question, whether or not a given mathematical model can admit more than one steady state. As parameter values (like rate constants and total concentrations) are often unknown or subject to a very high uncertainty due to measurement errors and difficult experimental conditions, one is often interested in the question, whether or not a given mathematical model can, for some conceivable parameter vector, exhibit multistationarity at all. A partial answer to this question is given in Feinberg's deficiency one algorithm. This algorithm can decide about the existence of multistationarity by analyzing a, potentially large, set of systems of linear inequalities that are independent of parameter values. However, the deficiency one algorithm is limited to what its author calls **regular deficiency one networks**. Many realistic networks have a deficiency higher than one, thus the algorithm cannot be applied directly. In a previous publication it was suggested to analyze certain well defined subnetworks that are guaranteed to be of deficiency one. If these subnetworks are regular, then one can use the deficiency one algorithm to establish multistationarity. Realistic reaction networks, however, often lead to subnetworks that are irregular, especially if metabolic networks are considered. Here the special structure of the subnetworks is used to derive conditions for multistationarity. These conditions are independent of the regularity conditions required by the deficiency one algorithm. Thus, in particular, these conditions are applicable to irregular subnetworks.

1 Introduction

In Systems Biology there is a growing interest in the question, whether or not a given mathematical model can admit more than one steady state. In cell

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cycle regulation, for example, one can identify different phases of the cell cycle (G1, S, G2 and M-phase) as different stable steady states. The cycle itself can then be considered as a switching between these steady states. As parameter values (like rate constants and total concentrations) are often unknown or subject to a very high uncertainty due to measurement errors and difficult experimental conditions, one is often interested in the question, whether or not a given mathematical model can, for some conceivable parameter vector, exhibit multistationarity at all.

A partial answer to this question is given in Feinberg's chemical reaction network theory, that links the ability of a mathematical model to exhibit multistationarity to the structure of the underlying biochemical reaction network [5, 6, 7, 8]. The deficiency one algorithm, in particular, can decide about the existence of multistationarity by analyzing a, potentially large, set of systems of linear inequalities that depend on the network structure alone, that is, that are independent of parameter values. If any of these inequality systems is feasible, then multistationarity is guaranteed and one can compute steady states and rate constants from its solution set. If all are infeasible, then multistationarity is impossible, for any conceivable parameter vector (see, for example, [5, 8]). Observe that, in particular, this algorithm can also be used to prove that multistationarity is impossible.

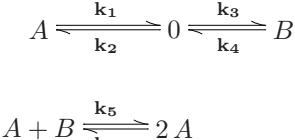
However, the deficiency one algorithm is limited to what its author calls **regular deficiency one networks** [8]. Many realistic networks have a deficiency higher than one, thus the algorithm cannot be applied directly. In [3, 9] we therefore suggested a way to circumvent this: instead of analyzing the complete network we propose to analyze certain well defined subnetworks that are guaranteed to be of deficiency one. If these subnetworks are regular, then one can use the deficiency one algorithm to establish multistationarity. If this is successful, then [3] gives sufficient conditions that are computationally simple to check to extend multistationarity from the subnetwork to the overall network.

Realistic reaction networks, however, often lead to subnetworks that are irregular, especially if metabolic networks are considered (see e.g. [10] for an analysis of the upper part of glycolysis). Consequently, the deficiency one algorithm cannot be applied to these subnetworks. If this irregularity is of a special kind (termed \emptyset -irregularity in [10]), then one can regularize the subnetwork and apply the deficiency one algorithm to the resulting regularized subnetwork. It is then possible to extend multistationarity – so it exists – to the overall network using the aforementioned results of [3].

Here we follow a different approach: instead of trying to regularize a subnetwork, we use the special structure of the subnetworks defined in [3] to derive conditions for multistationarity. These conditions are independent of the regularity conditions required by the deficiency one algorithm. Thus, in particular, these conditions are applicable to irregular subnetworks. Of course it is still possible to use the results of [3] to extend multistationarity (once it can be established in the subnetwork).

2 Notation

Consider the following (bio)chemical reaction network with $n = 2$ species A and B and with $m = 5$ complexes A , 0 , B , $A + B$ and $2A$ and $r = 6$ reactions:



Let $x \in \mathbb{R}^n$ be the vector of species concentrations (e.g. let x_1 be the concentration of A and x_2 be the concentration of B). By associating each concentration with the corresponding unit vector e_i of Euclidean space (A with e_1 and B with e_2 in case of the example) one can define m ‘complex’-vectors y_i (in case of the example $y_1 = e_1$ for A , $y_2 = 0$, the 2-dimensional zero vector for the complex 0 , $y_3 = e_2$ for B , $y_4 = e_1 + e_2$ for $A + B$ and $y_5 = 2e_1$ for $2A$). Collect these in a matrix $Y \in \mathbb{R}^{n \times m}$. For the example one obtains

$$\begin{aligned} Y &= [y_1 \quad \dots \quad y_5] \\ &= \begin{bmatrix} 1 & 0 & 0 & 1 & 2 \\ 0 & 0 & 1 & 1 & 0 \end{bmatrix}. \end{aligned}$$

Let I_a be the incidence matrix of the graph associated to the reaction network in standard form as defined in [7, 8], that is a graph, where node labels are unique. This means that one has $I_a \in \{-1, 0 - 1\}^{m \times r}$. For the example one obtains

$$I_a = \begin{bmatrix} -1 & 1 & 0 & 0 & 0 & 0 \\ 1 & -1 & -1 & 1 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 0 & 1 & -1 \end{bmatrix}$$

Finally let $k \in \mathbb{R}_{>0}^r$ be the vector of rate constants, that is for the example:

$$k = (k_1, \dots, k_6).$$

The stoichiometric matrix N is defined as the product

$$N := Y I_a \tag{1}$$

of the matrix of complexes Y and the incidence matrix of the associated directed graph I_a .

Definition 1 (Reactant Complex, Educt, \bar{m}). *A complex that has at least one outgoing edge is called a reactant complex. We use the symbol $\bar{m} \leq m$ to denote the number of reactant complexes.*

Let y be a reactant complex. Then all species with indices contained in $\text{supp}(y)$ are called educts.

For simplicity we assume – w.l.o.g. – the following ordering of complexes:

Assumption 2 (Complex Ordering). *Assume that the complexes are ordered such that the first \bar{m} complexes are reactant complexes.*

Under this assumption the mapping reac that associates every reaction with its reactant complex has a particular simple form:

Definition 3 (Mapping reac). *Let $\text{reac} : \{1, \dots, r\} \rightarrow \{1, \dots, \bar{m}\}$ be defined as*

$$\text{reac}(j) = i, y_i \text{ is the tail of reaction } j. \quad (2)$$

If mass-action kinetics is used, then the reaction rate $v_i(k, x)$ associated to the i -th reaction is given as the monomial $v_i(k, x) = k_i x^{y_{\text{reac}(i)}}$ (i.e. the reaction rate $v_i(k, x)$ is proportional to the product of the educt concentrations). One obtains the following function $v(k, x)$:

Definition 4 ($v(k, x)$, $\Phi(x)$, $\Psi(x)$). *Using mass action kinetics, the vector of reaction rates is defined as*

$$v(k, x) := \text{diag}(k) \Phi(x), \quad (3a)$$

where

$$\Phi(x) := (x^{y_{\text{reac}(1)}}, \dots, x^{y_{\text{reac}(r)}})^T. \quad (3b)$$

Let e_i denote the unit vectors of Euclidian n -space and define

$$\Pi := \begin{bmatrix} e_{\text{reac}(1)}^T \\ \vdots \\ e_{\text{reac}(r)}^T \end{bmatrix} \quad (3c)$$

$$\Psi(x) := (x^{y_i})_{i=1, \dots, \bar{m}}. \quad (3d)$$

Note that this implies that

$$\Phi(x) = \Pi \Psi(x) \quad (3e)$$

and thus

$$v(k, x) = \text{diag}(k) \Phi(x) = \text{diag}(k) \Pi \Psi(x) \quad (3f)$$

hold. Let

$$\hat{Y} = [y_i]_{i=1, \dots, \bar{m}} \quad (3g)$$

be a matrix having the exponents of $\Psi(x)$ as column vectors (recall assumption 2 and note that this implies that \hat{Y} contains the first \bar{m} columns of Y).

For the example one obtains

$$v(k, x) = (k_1 x_1, k_2, k_3, k_4 x_2, k_5, x_1 x_2, k_6 x_1^2)^T.$$

Observe that $\hat{Y} = Y$ and thus

$$\Phi(x) = \Psi(x),$$

in this case. Then the following system of Ordinary Differential Equations (ODEs) describes the dynamics of the species concentrations:

$$\dot{x} = N v(k, x), \quad (4a)$$

If the stoichiometric matrix $N \in \mathbb{R}^{n \times r}$ does not have full row rank, the system is subject to ‘conservation relations’: let $s = \text{rank}(N) < n$, then there is a matrix $W \in \mathbb{R}^{n \times n-s}$ with $W^T N = 0$ and

$$W^T x(t) = c \quad (4b)$$

along solutions $x(t)$ of (4a), cf. [1].

As we are mainly interested in positive steady states, the pointed polyhedral cone $\ker(Y I_a) \cap \mathbb{R}_{\geq 0}^r$ is of particular interest. The symbol E is used to denote the unique (up to scalar multiplication) generators of $\ker(Y I_a) \cap \mathbb{R}_{\geq 0}^r$. Let p be the number of generators, if $p > 1$, then $E \in \mathbb{R}^{r \times p}$ is a matrix whose columns are the generators of $\ker(Y I_a) \cap \mathbb{R}_{\geq 0}^r$, if $p = 1$, then $E \in \mathbb{R}^r$ is a vector. Define the set of all nonnegative vectors $x \in \mathbb{R}_{\geq 0}^p$ such that $E x$ is positive:

$$\Lambda(E) := \left\{ x \in \mathbb{R}_{\geq 0}^p \mid E x > 0 \right\}. \quad (5)$$

3 Some remarks about positive steady states

The structure of (4a) motivates the following result concerning positive steady states:

Lemma 1 (Existence of positive steady states). *Consider a system of ODEs as in (4a), with stoichiometric matrix N and let $E \in \mathbb{R}_{\geq 0}^{r \times p}$ be the generator matrix of $\ker(Y I_a) \cap \mathbb{R}_{\geq 0}^r$. Let $k \in \mathbb{R}_{>0}^r$ be given. Then the positive vector a is a solution to the polynomial equation $N v(k, a) = 0$, if and only if there exists a vector $\lambda \in \Lambda(E)$ with*

$$k = \text{diag}(\Phi(a^{-1})) E \lambda. \quad (6)$$

Proof. Follows from the fact that $a > 0$ and $k > 0$ implies $v(k, a) > 0$. Thus $N v(k, a) = 0$ holds if and only if $v(k, a) \in \ker(Y I_a) \cap \mathbb{R}_{\geq 0}^r$, that is, if and only if $v(k, a) = E \lambda$, for some $\lambda \in \Lambda(E)$. As $v(k, a) = \text{diag}(k) \Phi(a)$ (6) follows immediately. \square

Remark 1. If a positive steady state exists, then (6) must hold. The condition (6) can thus be used to constrain the set of rate constants that allow the existence (of at least one) positive steady state.

Remark 2 (Positive steady states). Consider a system of ODEs as in (4a) and let $E \in \mathbb{R}_{\geq 0}^{r \times p}$ be the generator matrix of $\ker(Y I_a) \cap \mathbb{R}_{\geq 0}^r$. The system has a positive steady state, iff $\ker(Y I_a) \cap \mathbb{R}_{\geq 0}^r \neq \emptyset$ and the rows of E are nonzero.

Remark 3. Consider a system of ODEs as in (4a) and let $E \in \mathbb{R}_{\geq 0}^{r \times p}$ be the generator matrix of $\ker(Y I_a) \cap \mathbb{R}_{\geq 0}^r$ and suppose that E does not contain any zero rows. Then every positive vector a can be a steady state of (4a), by choosing k as in (6), where $\lambda \in \Lambda(E)$ is free and takes the role of the rate constants.

4 Subnetworks defined by stoichiometric generators

In this section the following concepts from graph theory will be used (two of them are standard definitions in graph theory, that stated here merely for convenience, while the third, very common in CRNT, is derived from those two):

Definition 5. [11] *Connected component: the maximal connected subgraphs of a graph*

[11] *Strongly connected component: a directed graph is called strongly connected if there is a path from each vertex in the graph to every other vertex. The strongly connected components (SCC) of a directed graph are its maximal strongly connected subgraphs.*

[8] *Terminal strongly connected component: an scc that has no outgoing edge*

Next we recall some results concerning subnetworks defined by stoichiometric generators

Lemma 2 (Properties of subnetworks defined by stoichiometric generators). *For a subnetwork that is defined by a stoichiometric generator E the following properties hold:*

- (a) *Graph of the network in normal form is a forest of trees*
- (b) *Terminal strongly connected components consist of a single node (complex)*
- (c) *The deficiency of the network is one*
- (d) *The deficiency of every connected component is zero*
- (e) *If every connected component contains only one terminal strongly connected component, then the network is regular (in the sense of CRNT, cf.[4, 5], for example)*

(f) $\ker(N) = [E]$

Proof.

- (a),(b) Follow from the definition of the generators of $\ker(Y I_a) \cap \mathbb{R}_{\geq 0}^r$
- (c)-(f) A proof can be found in [3]

□

The following corollary is an immediate consequence of Lemma 2, (f) and Remark 3.

Corollary 1. *Consider a biochemical reaction network that is defined by a stoichiometric generator. Then*

- (i) *any positive vector a is a steady state of (4a), if k is chosen as in (6)*
- (ii) *for an arbitrary but fixed positive a , k as in (6) is fixed up to scalar multiplication (i.e. the positive λ)*
- (iii) *(positive) scalar multiplication of k corresponds to a time scaling of the ODEs, thus one can – w.l.o.g. – choose $\lambda = 1$*

From here on we assume that the system has at least one positive steady state, that is

Assumption 6. *The vector of rate constants is given by*

$$k = \text{diag}(\Phi(a^{-1})) E \quad (7)$$

for some $a \in \mathbb{R}_{>0}^n$.

Consider Lemma 1 and especially the facts that for networks defined by stoichiometric generators E consists of one (column) vector and that – w.l.o.g. – $\lambda = 1$. Then the ODEs (4a) are equivalent to

$$\dot{x} = N v(k, x) = N \text{diag}(E) \text{diag}(\Phi(a^{-1})) \Phi(x) = N \text{diag}(E) \Phi\left(\frac{x}{a}\right),$$

where $\Phi(x) = \Pi \Psi(x)$ (cf. Definition 4). Thus

$$\dot{x} = N \text{diag}(E) \Pi \Psi\left(\frac{x}{a}\right) = N \text{diag}(E) \Pi \text{diag}(\Psi(a^{-1})) \Psi(x) \quad (8)$$

follows.

Remark 4. *Systems like (8) are sometimes called generalized mass action systems. For those systems reaction rates $v_i(k, x)$ are still defined as monomials $k_i x^{y_{\text{reac}(i)}}$, however the exponent vector $y_{\text{reac}(i)}$ does not need to correspond to the reactant stoichiometry anymore.*

Further observe that for the special system defined in (8) $\Psi(a^{-1})$ takes the role of the rate constants.

To establish multistationarity we need to show the existence of a second steady state $b \in \mathbb{R}_{>0}^n$ with

$$N v(k, b) = 0,$$

for the **same vector** k . That is b must satisfy:

$$N \operatorname{diag}(E) \Pi \operatorname{diag}(\Psi(a^{-1})) \Psi(b) = 0 \quad (9)$$

Obviously $\ker(N \operatorname{diag}(E) \Pi) = [\underline{1}_{\bar{\rho}}]$ (as N is the stoichiometric matrix of a subnetwork defined by a stoichiometric generator; to see this recall that (i) $\ker(N) = [E]$ and (ii) Π has full column rank and (iii) row vectors of Π are unit vectors: thus $\operatorname{diag}(E) \Pi \underline{1}_{\bar{m}} = E$). It follows that (9) is equivalent to (observe that $a, b > 0$ implies $\Psi(\frac{b}{a}) > 0$):

$$\Psi\left(\frac{b}{a}\right) = \alpha \underline{1}_{\bar{m}}, \alpha > 0$$

Apply $\ln(\cdot)$ to obtain the *linear system*

$$\hat{Y}^T \mu = \ln(\alpha) \underline{1}_{\bar{m}}, \quad (10a)$$

where

$$\mu := \ln \frac{b}{a} = \left(\ln \frac{b_1}{a_1}, \dots, \ln \frac{b_n}{a_n} \right)^T. \quad (10b)$$

The previous discussion motivates the following Lemma:

Lemma 3 (Parameterizing positive steady state solutions). *Consider the ODEs derived from a biochemical reaction network that is defined by a stoichiometric generator. Let*

$$\mathcal{M} := \left\{ \mu \in \mathbb{R}^n \mid \exists \rho > 0, \text{ such that } \hat{Y}^T \mu = \rho \underline{1}_{\bar{m}} \right\}. \quad (11a)$$

If $\mathcal{M} \neq \emptyset$, then $\mu \in \mathcal{M}$ and $a \in \mathbb{R}_{>0}^n$ parameterize positive solutions of the polynomial equation $N v(k, b) = 0$. Let $\mu \in \mathcal{M}$ and let

$$k = \lambda \Phi(a^{-1}), \lambda > 0 \quad (11b)$$

be the vector of rate constants. Further let

$$b = \operatorname{diag}(e^\mu) a. \quad (11c)$$

Then $N v(k, a) = 0$ and $N v(k, b) = 0$ hold.

Proof. Let $\mu \in \mathcal{M}$ let k and b be as in (11b), (11c), respectively. Observe that $N v(k, a) = 0$ follows from Lemma 1. Thus we have to show that $N v(k, b) = 0$

holds. To this end observe that

$$\begin{aligned}
N v(k, b) &= N \operatorname{diag}(\lambda E) \operatorname{diag}(\Phi(a^{-1})) \Phi(b) \\
&= \lambda N \operatorname{diag}(E) \operatorname{diag}(\Phi(a^{-1})) \Phi(\operatorname{diag}(e^\mu) a) \\
&= \lambda N \operatorname{diag}(E) \operatorname{diag}(\Phi(a^{-1})) \operatorname{diag}(\Phi(a)) \Phi(e^\mu) \\
&= \lambda N \operatorname{diag}(E) \Pi \Psi(e^\mu) \\
&= \lambda N \operatorname{diag}(E) \Pi e^{\hat{Y}^T \mu} \\
&= \lambda N \operatorname{diag}(E) \Pi e^\rho \underline{1}_{m_1} = 0.
\end{aligned}$$

□

Remark 5. If either (i) $\underline{1}_{\bar{m}} \in [\hat{Y}^T]$ or (ii) $\ker(\hat{Y}^T)$ is nontrivial (i.e. $\operatorname{rank}(\hat{Y}) < n$), or both, then a fixed vector $a \in \mathbb{R}_{>0}^n$ together with k as in (11b) defines an infinite set of positive steady states $b \in \mathbb{R}_{>0}^n$. To see this assume that (i), (ii) or both hold. Then (10a) is solvable and the solution set

$$\mathcal{M} := \left\{ \mu \in \mathbb{R}^n \mid \exists \rho > 0, \text{ such that } \hat{Y}^T \mu = \rho \underline{1}_{\bar{m}} \right\}$$

defines a linear subspace, that is there exists a matrix M and a vector κ of appropriate dimensions, such that

$$\mu \in \mathcal{M} \Leftrightarrow \mu = M \kappa$$

holds. As every $\mu \in \mathcal{M}$ defines a positive b and as a linear vector space contains infinitely many elements μ , there exists, for a fixed $a \in \mathbb{R}_{>0}^n$ infinitely many $b \in \mathbb{R}_{>0}^n$ with

$$b_a(\kappa) = \operatorname{diag}(e^{M\kappa}) a. \quad (12)$$

5 Subnetwork multistationarity

If the conditions of Remark 5 hold, then there exists an infinite set of positive steady states, even for a given $a \in \mathbb{R}_{>0}^n$ (recall that $\Psi(a^{-1})$ takes the role of the rate constants). Fix $a \in \mathbb{R}_{>0}^n$ and thus $k = \operatorname{diag}(\Phi(a^{-1})) E = \operatorname{diag}(E) \Pi \Psi(a^{-1})$. Then all $b_a(\kappa)$ as defined in (12) are steady states. However, for a given initial condition $x_0 \in \mathbb{R}_{>0}^n$ the system ‘sees’ only a subset of set of positive steady states.

To see this recall the ODEs derived from a biochemical reaction network $\dot{x} = N v(k, x)$ and let $s := \operatorname{rank}(N) < n$. Let S, W be orthonormal bases of $[N] =: \mathcal{S}$ and \mathcal{S}^\perp , respectively. Similar to [1], introduce the transformation

$$y = S^T x, z = W^T x \text{ and } x = \xi(x, y) = S y + W z.$$

In the new coordinates the ODEs read as

$$\begin{aligned}
\dot{y} &= S^T \dot{x} = S^T N \operatorname{diag}(E) \Pi \operatorname{diag}(\Psi(a^{-1})) \Psi(\xi(y, z)) \\
\dot{z} &= W^T \dot{x} = 0
\end{aligned}$$

showing the invariance of \mathcal{S} . Let $x(0) = x_0 \in \mathbb{R}_{>0}^n$, then the solution $x(t)$ is given by

$$x(t) = x_0 + \int_0^t N \operatorname{diag}(E) \Pi \operatorname{diag}(\Psi(a^{-1})) \Psi(x(\tau)) d\tau.$$

For the new coordinates one obtains

$$\begin{aligned} y(t) &= S^T x_0 + S^T \int_0^t N \operatorname{diag}(E) \Pi \operatorname{diag}(\Psi(a^{-1})) \Psi(x(\tau)) d\tau \\ z(t) &= \tilde{W}^T x_0 = \text{const.} \end{aligned}$$

that is solutions are confined to parallel translates of \mathcal{S} . Thus, for a given initial condition, the system ‘sees’ only those positive steady states that are in the intersection of $b_a(\kappa)$ and \mathcal{S} . Observe that $\tilde{\mathcal{S}} := [N \operatorname{diag}(E) \Pi] \subseteq \mathcal{S}$ and that, by a similar argument, solutions are confined to parallel translates of $\tilde{\mathcal{S}}$. This motivates the following definition of multistationarity with respect to a linear subspace as introduced in [9]:

Definition 7. Given a subspace $\mathcal{V} \subset \mathbb{R}^n$, the system $\dot{x} = N v(k, x)$ from (4a) with stoichiometric subspace $\mathcal{S} = \operatorname{im}(N)$ is said to exhibit \mathcal{V} -multistationarity if and only if there exist a positive vector $k \in \mathbb{R}_{>0}^r$ and at least two distinct positive vectors $a, b \in \mathbb{R}_{>0}^n$ with

$$N v(k, a) = 0, \quad N v(k, b) = 0, \tag{13a}$$

$$b - a \in \mathcal{V}. \tag{13b}$$

Remark 6. Note that if $\mathcal{V} = \mathcal{S}$, then Definition 7 is equivalent to the familiar definition of multistationarity in Chemical Engineering and especially in CRNT as defined, for example, in [5, 8].

Remark 7. Note that for subnetworks defined by stoichiometric generators two linear subspaces are of particular interest: $[N \operatorname{diag}(E) \Pi]$ and the image of stoichiometric matrix of the overall network $[\hat{N}]$. Multistationarity with respect to $[N \operatorname{diag}(E) \Pi]$ means that the subnetwork can exhibit multistationarity, if it is considered in isolation, while multistationarity with respect to $[\hat{N}]$ means that the subnetwork as part of the larger network can exhibit multistationarity. Thus, if a subnetwork exhibits $[\hat{N}]$ -multistationarity, but not $[N \operatorname{diag}(E) \Pi]$ -multistationarity, then this subnetwork can give rise to multistationarity for the overall network, even though, in isolation, it does not exhibit multistationarity.

Remark 8. As an illustration consider the network in Fig.1(a). For this network the matrix N is given by

$$N = \begin{bmatrix} 1 & 0 & -1 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix} \tag{14a}$$

the unique generator of $\ker(Y I_a) \cap \mathbb{R}_{\geq 0}^r$ is given by

$$E = (1, 1, 1, 1)^T. \quad (14b)$$

For $\Phi(x)$ one obtains

$$\Phi(x) = (1, 1, x_3, x_1 x_2)^T \quad (14c)$$

and therefore

$$\Pi = \begin{bmatrix} 1 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \quad \Psi(x) = (1, x_3, x_1 x_2)^T. \quad (14d)$$

Thus one obtains

$$k = \left(1, 1, \frac{1}{a_1 a_2}, \frac{1}{a_3} \right)^T \quad (14e)$$

for arbitrary $a > 0$. Observe that for this example $N \operatorname{diag}(E) \Pi = N \Pi$. It is straightforward to verify that all points on the following one-dimensional curve are steady states (parameterized by $p > 0$):

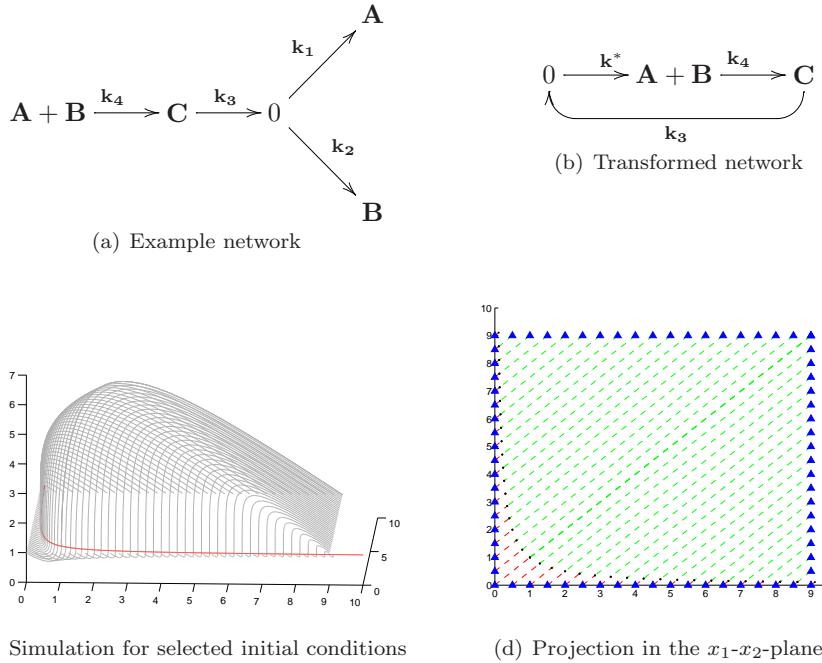
$$x_s(p) = \left(p, \frac{1}{p}, 1 \right). \quad (14f)$$

As N has full row rank, the left kernel is trivial, that is, it is spanned by $W = 0$, the three-dimensional zero vector. Pick two distinct real numbers $p_1 > p_2 > 0$. Then $x_s(p_1), x_s(p_2)$ are steady states that satisfy $W^T(x_s(p_1) - x_s(p_2)) = 0$ (i.e. $x_s(p_1) - x_s(p_2) \in [N]$). Thus, according to our definition, the system exhibits $[N]$ -multistationarity.

It fails to exhibit $[N \Pi]$ -multistationarity, as for a given initial condition $x_0 > 0$, all trajectories converge to a unique steady state. That is due to the fact that

$$N \Pi = \begin{bmatrix} 1 & -1 & 0 \\ 1 & -1 & 0 \\ 0 & 1 & -1 \end{bmatrix}$$

has a nontrivial left kernel $\tilde{W} = (1, -1, 0)^T$. Thus, for a given initial condition, a trajectory is confined to an affine linear subspace perpendicular to \tilde{W} . And all trajectories starting in particular affine linear subspace converge to the same steady state (demonstrated numerically for $a = 1$ in Fig.1(c) and 1(d)). Note that, using k as in (14e) the ODEs are equivalent to a system of ODEs derived from the network displayed in Fig.1(b), a weakly reversible deficiency zero network. From the Deficiency Zero Theorem [4] follows that this network



has a unique, asymptotically stable positive steady state – relative to a given initial condition.

Further note that the network in Fig. 1(a) is irregular in the sense of CRNT (cf. [5]) and that it fails to exhibit $[N \Pi]$ -multistationarity, while it exhibits $[N]$ -multistationarity.

6 Establishing multistationarity for subnetworks

Consider a biochemical reaction network defined by a stoichiometric generator. From Section 4 it is known, that for a given $a \in \mathbb{R}_{>0}^n$ all points $b_a(\kappa)$ as defined in (12) are steady states. Moreover, the set \mathcal{M} , a linear subspace, as defined in (11a) contains all $\mu = \ln b_a(\kappa) - \ln a$. From Section 5 it is known, that \mathcal{V} -multistationarity requires

$$\begin{aligned} b_a(\kappa) - a &\in \mathcal{V} \\ \ln b_a(\kappa) - \ln a &\in \mathcal{M} \end{aligned}$$

To this end a result from [2] can be used. To state the result, let $\text{sign}(u)$ denote the sign pattern of the vector $u \in \mathbb{R}^n$. Then $v = \text{sign}(u)$ is a vector with entries $v_i \in \{+, -, 0\}$ depending on whether $u_i > 0$, $u_i < 0$ or $u_i = 0$, respectively.

Lemma 4 (cf. [2]). Let $M_1 \subseteq \mathbb{R}^n$ and $M_2 \subseteq \mathbb{R}^n$ be two nontrivial subsets of \mathbb{R}^n and define $M_3 := \{(m_1, m_2) \in M_1 \times M_2 \mid \text{sign}(m_1) = \text{sign}(m_2)\}$ as the set of all ordered pairs (m_1, m_2) of elements $m_1 \in M_1$ and $m_2 \in M_2$ with the same sign pattern. Two positive vectors p and q with $\ln q - \ln p \in M_1$ and $q - p \in M_2$ exist, if and only if $M_3 \neq \emptyset$. Then p and q are given by

$$(p_i)_{i=1, \dots, n} = \begin{cases} \frac{m_{2i}}{e^{m_{1i}} - 1}, & \text{if } m_{1i} \neq 0 \\ \bar{p}_i > 0, & \text{if } m_{1i} = 0, \end{cases} \quad (15)$$

where \bar{p}_i denotes an arbitrary positive number and

$$(q_i)_{i=1, \dots, n} = e^{m_{1i}} p_i. \quad (16)$$

Thus – using $b_a(\kappa)$ instead of q , a instead of p and \mathcal{M} instead of M_1 , \mathcal{V} instead of M_2 – all one has to do is to find a vector $\mu \in \mathcal{M}$ and a vector $s \in \mathcal{V}$ with $\text{sign}(\mu) = \text{sign}(s)$ to establish \mathcal{V} -multistationarity.

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